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Mitochondrial electron transport and glycolysis are coupled in articular cartilage

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SUMMARY

Objective: Although the majority of the adenosine triphosphate (ATP) in chondrocytes is made by glycolysis rather than by oxidative phosphorylation in mitochondria there is evidence to suggest that reactive oxygen species produced by mitochondrial electron transport (ET) help to maintain cellular redox balance in favor of glycolysis. The objective of this study was to test this hypothesis by determining if rotenone, which inhibits ET and blocks oxidant production inhibits glycolytic ATP synthesis.
Design: Bovine osteochondral explants were treated with rotenone, an ET inhibitor; or oligomycin an ATP synthase inhibitor; or 2-fluoro-2-deoxy-D-glucose, a glycolysis inhibiter; or peroxide, an exogenous oxidant; or mitoquinone (MitoQ), a mitochondria-targeted anti-oxidant. Cartilage extracts were assayed for ATP, nicotine adenine dinucleotide (NAD+/H), and culture medium was assayed for pyruvate and lactate after 24 h of treatment. Imaging studies were used to measure superoxide production in cartilage.
Results: Rotenone and 2-FG caused a significant decline in cartilage ATP ($P < 0.001$). In contrast, ATP levels were not affected by oligomycin. Peroxide treatment blocked rotenone effects on ATP, while treatment with MitoQ significantly suppressed ATP levels. Rotenone and 2-FG caused a significant decline in pyruvate, but not in lactate production. NADH:NAD+ ratios decreased significantly in both rotenone and 2-FG-treated explants ($P < 0.05$). Rotenone also significantly reduced superoxide production.
Conclusions: These findings showing a link between glycolysis and ET are consistent with previous reports on the critical need for oxidants to support normal chondrocyte metabolism. They suggest a novel role for mitochondria in cartilage homeostasis that is independent of oxidative phosphorylation.

Introduction

Biosynthetic activity required for the maintenance and repair of the articular cartilage extracellular matrix (ECM) draws heavily on chondrocyte energy stores and ECM synthesis rates are closely tied to the availability of adenosine triphosphate (ATP)[1–3]. ECM synthesis increases when cartilage is damaged by mechanical injury, increasing the demand for ATP[4]. Thus, inadequate ATP production has the potential to undermine repair activities that counter cartilage degeneration[5,6].

Articular cartilage chondrocytes harbor relatively few mitochondria compared with most other cell types[7] and may not express all subunits of the mitochondrial electron transport (ET) chain[8]. Moreover, ET is impaired by low oxygen levels in the cartilage matrix[1]. As a result, less than 10% of total cellular ATP is derived from oxidative phosphorylation and the remainder is produced largely *via* the Embden-Meyerhof glycolysis pathway[9]. Nevertheless, mitochondrial damage and dysfunction has been implicated in primary and post-traumatic osteoarthritis[10–13], indicating that mitochondria play important roles in cartilage physiology despite their minimal ATP-generating capacity. However, precisely how mitochondria function in chondrocyte metabolism is still somewhat obscure.

Our recent work showed that mitochondria are a principle source of reactive oxygen species released by chondrocytes in response to blunt impact injuries[13]. That study showed that treatment with rotenone, an inhibitor of complex I of the mitochondrial ET chain, prevented reactive oxygen species (ROS) release and chondrocyte death after impact. Although in that context ROS were produced at lethal levels, it remains possible that at lower levels, ROS produced by mitochondrial might play a role in normal physiology. In cartilage, ROS have been extensively studied in terms of their role in the pathogenesis of OA; however, low, sub-lethal ROS levels appear to have beneficial effects and are important for cartilage homeostasis[14,15]. Extracellular superoxide and hydrogen peroxide activate the mitogen activated kinases extracellular

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regulated kinase (ERK) 1 and 2 and janus kinase (JNK), which regulate catabolic and anabolic gene expression[15]. Treatment of chondrocytes with sub-lethal doses of hydrogen peroxide suppressed interleukin-1- and lipopolysaccharide-induced increases in the expression of pro-inflammatory mediators such as nitric oxide synthase[16]. Furthermore, cartilage explants conditioned by repeated peroxide treatment were protected from apoptosis and other harmful effects of mechanical compression through up-regulation of catalase gene expression and down regulation of matrix metalloprotease-3[17]. While nicotine adenine dinucleotide phosphate (NADPH) oxidase has been thought to be the primary source of superoxide in cartilage[15], our work suggests that mitochondria also play an important role.

Results published by Lee and Urban revealed that oxygen or other electron acceptors support glycolysis in articular cartilage. Their initial findings showed a negative Pasteur Effect whereby ATP and lactate production in cartilage declined sharply under anoxic conditions[1]. Subsequently they showed that anaerobic glycolysis was rescued by providing exogenous oxidants, which appeared to maintain redox balance in chondrocytes exposed to anoxic or severely hypoxic conditions[18]. In the light of these studies we propose that ROS released by mitochondria might constitute a natural source of oxidants that supports glycolytic ATP synthesis in chondrocytes. This mechanism might be particularly important following joint injury, when damage to the cartilage matrix stimulates demand for *de novo* matrix production at a time when ambient oxygen levels have dramatically declined[19].

Based on the findings summarized above we hypothesized that cytosolic glycolysis and mitochondrial ET are coupled. To test this, we measured tissue-level ATP, nicotine adenine dinucleotide (NAD+/H), and pyruvate levels in bovine cartilage treated with inhibitors of glycolysis, ET, and oxidative phosphorylation. Osteochondral explants were treated with rotenone which inhibits mitochondrial ET at complex I (NADH dehydrogenase)[20]; or with oligomycin, which inhibits mitochondrial oxidative phosphorylation at complex V (ATP synthase)[21], or with 2-fluoro-2-deoxy-D-glucose (2-FG) which inhibits hexokinase at the initial step of glycolysis, or with mitoquinone (MitoQ), a free radical scavenger targeted to mitochondria[22–24]; or with tertiary butyl hydroperoxide (tBHP), a stabilized form of hydrogen peroxide. Effects of the inhibitors on cell viability and the effects of rotenone on superoxide production were also measured.

Methods

Metabolic inhibitors including rotenone, oligomycin, and 2-fluoro-2-deoxy-glucose and tBHP were purchased from Sigma Chemicals (St Louis, MO). MitoQ was a kind gift from Dr. Michel Murphy via Dr. Douglas Spitz of the University of Iowa Department of Radiology. Dihydroethidium (DHE) and calcein AM were purchased from Invitrogen (Carlsbad, CA). Bovine stifle joints were obtained from a local abattoir. Osteochondral explants (2.5 cm × 2.5 cm) were excised from the tibial plateau and placed immediately in Hanks balanced salt solution (HBSS). Explants were then transferred to low glucose culture medium (45% low glucose Dulbecco's Modified Eagle's Medium (DMEM), 45% Ham's F-12, 10% fetal calf serum, 0.1% Fungizone, 0.05% Pen-Strep) and incubated at 37°C in an atmosphere consisting of 5% oxygen, 80% nitrogen, and 5% carbon dioxide.

Following excision and 24 h of incubation in culture medium, three experimental groups of were treated with one of the three metabolic inhibitors at doses previously shown to be effective: 0.625, 2.5, 10 μM rotenone[13], 2.5, 5, 10 μM oligomycin[25], or 0.5, 1, 3 mM 2-FG[13,26,27]. A fourth experimental group was treated with a combination of 2-FG (1 mM) and oligomycin (5 μM). A fifth group was treated with MitoQ (4 μM). A sixth group was treated with tBHP alone (250 μM) and a seventh group was treated with a combination of tBHP (250 μM) and rotenone (2.5 μM). Treatments were continued for 24 h prior to metabolite assays. Six untreated explants served as controls.

The method for measuring ATP production in bovine chondrocytes was adapted from a method used to quantify ATP concentrations in spermatozoa[28]. Directly following harvest, 6-mm cartilage plugs were weighed (40–60 mg) and placed in 0.5 ml water containing a phosphatase inhibitor cocktail (sodium vanadate, sodium molybdate, sodium tartrate, and imidazole) diluted 1:100 as directed by the manufacturer (Sigma P5726). The tubes containing cartilage plugs were then placed into boiling water for 10 min to extract ATP. The extract was centrifuged (12,000 g, 5 min) to pellet debris. ATP levels in supernatants were assayed using a commercial kit (Sigma FLAA ATP Bioluminescent assay kit). The luminescence of samples and standards was measured using a 1450 MicroBeta TriLux Microplate Scintillation and Luminescence Counter. Each sample or standard was assayed in triplicate. Results are reported in nmol ATP/mg cartilage.

In separate specimens, the effects of the metabolic inhibitors on cell viability were assessed after a 24 h treatment period. Calcein AM (1 mM) was used to stain viable cells and ethidium homodimer-2 (1 mM) was used to detect dead cells (Invitrogen Molecular Probes) as previously described[29]. Samples were scanned to a depth of 200 μm in 20 μm intervals. At each 20 μm interval, viable (green) and nonviable (red) chondrocytes were counted. Image J software was used to determine the average percentage of viable cells for each of the metabolic inhibitor treatment groups.

DHE was used as a probe for superoxide production as described previously[13]. Briefly, untreated explants or explants treated with 2.5 mM rotenone for 2 h were stained with DHE and calcein AM (Invitrogen) for 30 min. The explants were scanned with a BioRad 1024 confocal microscope (BioRad, CA). Images were analyzed using a custom computer algorithm to count DHE and Calcein AM − positive cells in three 20× fields on each explant. Results are reported as the percentage of DHE positive cells (DHE stained/DHE-stained + Calcein AM stained).

NAD+ and NADH were measured using a fluorometric assay kit (Fluoro NAD) according to the supplier's protocol (Cell Technology Inc.). Two experimental groups (six explants per group) were treated for 24 h with either rotenone or 2-FG. Six untreated explants served as controls. Cartilage plugs (6 mm diameter, ~2 mm-thick) were bisected and each half weighed (20–30 mg). After rinsing in cold phosphate buffered saline the samples were chopped in cold NAD or NADH lysis buffer (200 μl) and homogenized using a Polytron device with a 5 mm standard generator. The homogenates were heated to 60°C for 15 min then cooled to 4°C. One-hundred μl of the reaction buffer was added followed by 200 μl of the opposite extraction buffer to neutralize the homogenates. The tubes were vortexed and centrifuged (8000 g) for 5 min. 50 μl of each sample supernatant or standard were mixed with 100 μl reaction cocktail in a black-bottomed 96-well plate (Nunc). A SpectraMax M5 microplate fluorometer (Molecular Devices) was used to measure fluorescence intensity (Excitation 565 nm, Emission 600 nm). Each sample was assayed in triplicate and the results are reported as the ratio of NADH to NAD+.

Pyruvate and lactate were measured in explant culture media using fluorometric assay kits (BioVision) according to the manufacturer's protocol. Experimental samples and untreated control explants were incubated in 10 ml culture medium (45% low glucose, pyruvate-free DMEM, 45% Ham's F-12, 10% fetal calf serum, 0.1% Fungizone, 0.05% Pen-Strep) for 24 h. Medium samples were processed by filtration using Microcon 3 kDa cutoff filters (Millipore). Samples and lactate or pyruvate standards (50 μl) were mixed with 50 μl reaction buffer in a black-bottomed 96-well

microplate. After a 30 min incubation at room temperature fluorescence intensity was measured in a SpectraMax M5 microplate fluorometer (excitation 535 nm, emission 570 nm). Results are reported in mmol/L (mM). Each sample was assayed in triplicate.

Oxygen saturation levels were measured using a Thermo Scientific biochemical oxygen demand (BOD) Probe. One gram of bovine articular cartilage obtained from the femoral condyle was used in each experiment. One bovine cheek muscle sample obtained from the same abbatoir was also examined. Cartilage samples were allowed to equilibrate to culture conditions (37°C, 5% CO_2) in the previously described culture medium for 24 h. Muscle samples were not allowed to equilibrate because of the rapid spoilage rate of the tissue in culture conditions. Prior to testing, the BOD probe was equilibrated to culture conditions for two h. Power was applied to the probe for 1 h of this period to allow for complete polarization. After 24 h of equilibration, samples were placed in a BOD bottle (Hach Company) in 10 ml of culture medium with treatment agents (if applicable), the probe was placed in the bottle, and the bottle sealed with two layers of Parafilm Laboratory Film (American National Can). The blank control sample was tested with only 10 ml of culture media present in the bottle. Oxygen saturation levels in the sealed bottle were read every 10 min for a total of 22 h using an accumet® excel XL40 Dissolved Oxygen Meter (Fisher Scientific). Oxygen saturation levels are presented as a percentage of initial oxygen concentration in the bottle.

Each explant was from a different animal. These were randomly assigned to a treatment groups using a uniform random number generator (SigmaPlot, Systat Software, San Jose CA). The means and C.I.s reported are based on one measurement per explant. One-way analysis of variance (ANOVA) on ranks with a *post hoc* Dunn's test for multiple comparisons was used for statistical analysis.

Results

The effects of various inhibitors on ATP are summarized in Table I. Untreated control samples produced near 0.7 nmol ATP/mg of tissue which decreased to varying degrees with different doses of rotenone or oligomycin. These effects were significant for rotenone and for 2-FG at the two highest doses. Oligomycin tended to increase ATP but the effects were not statistically significant. A combination of oligomycin and 2-FG gave similar results as 2-FG alone, indicating that the ATP remaining in the tissue when glycolysis was blocked was not generated by complex V, the mitochondrial ATPase inhibited by oligomycin. The mitochondria-targeted anti-oxidant MitoQ had a significant suppressive effect comparable in magnitude to that of rotenone, a result that shows a link between mitochondrial oxidants and ATP. Like oligomycin, the oxidant tBHP caused a slight increase that was not significant at the dose tested. However, tBHP appeared to block the inhibitory effect of rotenone, indicating that an exogenous oxidant can be substituted for oxidants produced by ET. At the dosages used in this study none of the inhibitors were found to be cytotoxic: Cell viability percentages in control specimens and the three treatment groups were all approximately 90% (Table II).

Table I
Effects of metabolic inhibitors on the ATP content of articular cartilage. ATP (nmol/mg) was measured in cartilage from explants treated for 24 h with various drugs at the concentrations indicated. Group means, confidence intervals (C.I.), and *P* values are shown. Each group is represented by five explants

	Dose (μM)	Mean	C.I.		*P* value
			High	Low	
Control	0	0.695	0.766	0.625	NA
Rote	0.625	0.439	0.560	0.318	0.054
	2.5	0.166	0.208	0.124	0.001
	10	0.176	0.211	0.141	0.001
2-FG	0.5	0.436	0.631	0.241	0.017
	1	0.085	0.104	0.066	0.001
	3	0.037	0.045	0.029	0.001
Oligo	2.5	0.725	1.061	0.389	0.832
	5	1.144	1.347	0.941	0.001
	10	0.626	0.744	0.508	0.584
Oligo/2-FG	5/1	0.107	0.118	0.096	0.001
MitoQ	4	0.201	0.234	0.168	0.001
tBHP	250	0.898	1.049	0.747	0.081
tBHP/Rote	250/2.5	0.947	1.103	0.791	0.037

Table II
Effects of rotenone and 2-FG on lactate and pyruvate production in explants. Lactate and pyruvate accumulation in culture media over 24 h was measured. Means (nmol/mg) confidence intervals (C.I.) are shown for lactate and pyruvate accumulation in culture media over 24 h. *P* values are indicated. $n = 5$ explants per group

	Viable (%)	C.I		*P* value
		Low	High	
Control	90	85	94	NA
Rotenone	87	81	93	0.58
Oligomycin	88	82	93	0.844
2-FG	90	84	96	0.448

The effects of rotenone (2.5 μM) on NAD+ and NADH levels were compared with the effects of 2-FG (1 mM) (Table III). Rotenone reduced NADH levels by more than 4-fold compared with controls while 2-FG reduced levels by more than 2-fold. Both drugs caused significant increases in NAD+ (1.5 and 2.5-fold for rotenone and 2-FG respectively). The NADH:NAD+ ratio in untreated controls was greater than 4, indicating that the bulk of the NAD in the cells was in the reduced form (Fig. 1). Direct glycolysis inhibition with 2-FG treatment reduced the NADH:NAD+ ratio more than 2-fold to less than 1.7, confirming the central role of glycolysis in the production of intracellular NADH from NAD^+. Rotenone treatment caused a five-fold decrease in the NADH:NAD+ ratio. To some degree, inhibitor effects on pyruvate production mirrored their effects on NADH and NAD+ (Table IV). Pyruvate concentrations in control explant media averaged over 1.0 mM, compared to 0.39 and 0.45 mM respectively in rotenone- and 2-FG-treated explants. In contrast, 2-FG and rotenone had no significant effects on lactate levels (Table IV).

The effects of rotenone on superoxide production were assessed using DHE as a probe (Fig. 2). Treatment with 2.5 μM rotenone reduced the number of cells stained with DHE from 9.3% to 4.2%. This effect was significant by *t*-test ($P = 0.019$). Oxygen consumption is expected to decline with oligomycin treatment, a consequence of the eventual inhibition of ET as protons are prevented from re-entering the mitochondrial matrix. To test for these effects in our system we compared O_2 consumption in untreated cartilage and in cartilage treated with 5 μM oligomycin (Fig. 3). Oligomycin

Table III
Effects of rotenone and 2-FG treatment on NADH and NAD levels. The NAD+/NADH content of tissue extracts was measured after 24 h treatments. Means (nmol/g) and confidence intervals (C.I.) and *P* values are given for each group. $n = 4$ explants per group

		Mean (nmol/g)	C.I.		*P* value
			Low	High	
NADH	Control	4.029	3.425	4.633	N/A
	2-FG	1.659	1.243	2.075	0.001
	Rotenone	0.639	0.518	0.760	0.001
NAD+	Control	0.956	0.794	1.118	N/A
	2-FG	2.586	2.406	2.766	0.001
	Rotenone	1.507	0.962	2.052	0.049

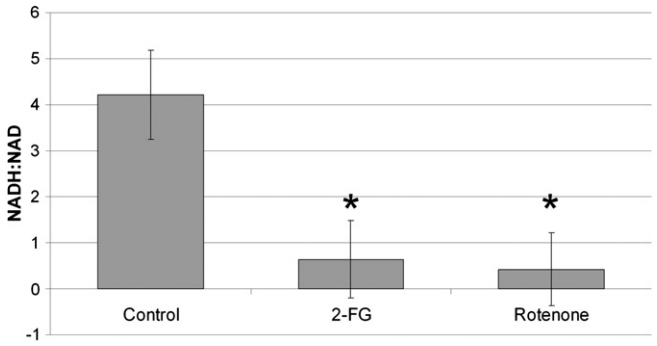

Fig. 1. Rotenone and 2-FG shifted the balance between the reduced and oxidized forms of NAD. Treatment with rotenone or 2-FG caused highly significant reductions in NADH:NAD+ ratios relative to untreated controls (*$P = 0.001$). There was no difference between the rotenone and 2-FG groups ($P = 0.749$). Means +/− confidence intervals (error bars) are based on four explants per group.

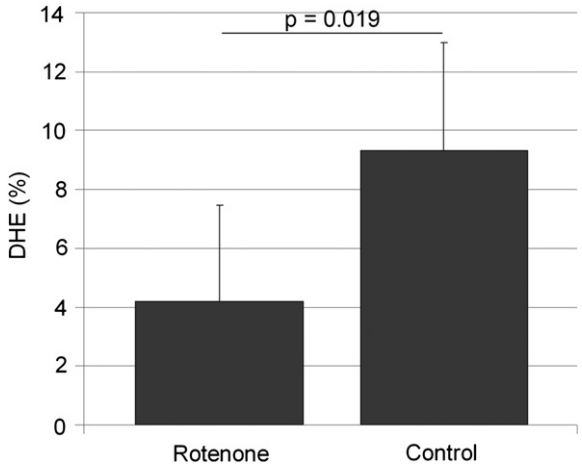

Fig. 2. Rotenone blocks oxidant production in cartilage. The graph shows the percentage of DHE staining chondrocytes in control explants and in explants treated with rotenone (2.5 μM). Means, confidence intervals C.I. (error bars), and *P* value are based on four explants per group.

completely blocked the normal O_2 consumption observed in control samples for up to 12 h. Thereafter there was incomplete recovery to near control values. These data confirm that oligomycin was effective in blocking complex V activity in our experiments.

Discussion

Tissue-level ATP levels were assayed to determine chondrocyte energy production from cells in their native matrix. If anything, blocking oxidative phosphorylation by inhibiting ATP-synthase with oligomycin had a slightly stimulatory effect on cartilage ATP levels. Oxygen consumption experiments, which showed that consumption was markedly reduced by oligomycin, confirmed the activity of the drug as used in our explant model. In contrast, inhibiting hexokinase at the initial step of glycolysis with 2-FG reduced ATP levels by up to several fold. Adding oligomycin to specimens already treated with 2-FG resulted in no further decreases in ATP, suggesting that the ATP remaining in cells treated with 2-FG was not produced by oxidative phosphorylation. *In situ* viability analysis revealed that none of the treatments were cytotoxic, confirming that their effects on ATP content were due to metabolic inhibition. These results agreed with those of other investigators, which show that inhibitors of mitochondrial oxidative phosphorylation, including uncouplers such as dinitrophenol have minimal effects on ATP pools in cartilage[1,30,31].

Given that neither oligomycin nor proton gradient uncouplers such as dinitrophenol cause significant depletion of cartilage ATP, rotenone's ability to deplete ATP and other metabolites was surprising. ATP, pyruvate, and NADH:NAD+ ratio in specimens treated with rotenone were significantly reduced nearly as much as with 2-FG treatment. The large difference in NADH:NAD+ ratios between rotenone-treated explants and controls was due to lower NADH levels together with higher NAD+ levels in treated the explants. These data are partially consistent with inhibition of glycolysis, which could plausibly deplete NADH. However it is unclear why NAD+ levels increased above control levels with rotenone, which blocks NADH dehydrogenase.

Assays revealed no significant treatment effects on lactate levels in this system. This together with the significant suppression of pyruvate by the drugs suggests that a greater proportion of the available pyruvate was converted to lactate in treated cells than in controls.

Our recent finding that rotenone blocks superoxide generation by chondrocytes in injured cartilage[13], and the findings reported here that superoxide production is reduced by rotenone in non-injured explants suggests that ET might influence glycolysis by contributing oxidants that function in intracellular signaling or in redox regulation. To investigate this hypothesis further we treated explants with MitoQ, a cationic free radical scavenger that is targeted to mitochondria[22]. This resulted in suppression of ATP to levels seen with rotenone treatment. On the other hand, we found that hydrogen peroxide blocked rotenone-induced reductions in ATP, indicating that exogenous oxidants can substitute for free radicals from ET. Although more definitive studies are needed, these findings support the hypothesis that ET's effects on glycolysis are mediated by free radicals emitted by the ET chain. Interestingly, the slight stimulatory effect of oligomycin, while difficult to explain in the light of its effectiveness at blocking oxygen consumption, might be attributable to its stimulation of ET-related free radical production[32].

In addition to NADPH oxidase, the mitochondrial ET chain appears to be a primary source of ROS in chondrocytes[33,34]. Superoxide is generated by high-energy electrons that escape from a series of bound complexes within the inner mitochondrial membrane. Complexes I and III of the ET chain are the most active superoxide generating sites and production from both sites is significantly reduced by Complex I inhibitors such as rotenone[35,36]. ROS from mitochondrial act as physiologic transduction signals[37–40]. Milner *et al.* reported that ROS generated mainly by Complex III regulate intracellular pH through effects on a Na^+/H^+ transporter[34]. In human umbilical vein endothelial cells subjected to repetitive cyclical strain, concentrations of mitochondrial ROS increased significantly together with robust increases in the expression of several cell adhesion markers. These responses were

Table IV
Effects of metabolic inhibitors on chondrocyte viability. Means, confidence intervals C.I. (error bars), and *P* values for each group are based on three explants. Viability was not significantly affected by treatment with any of the metabolic inhibitors

		Mean (mM)	C.I.		*P* value
			Low	High	
Pyruvate	Control	1.095	0.899	1.291	N/A
	2-FG	0.392	0.344	0.440	0.001
	Rotenone	0.447	0.287	0.607	0.001
Lactate	Control	0.065	0.041	0.089	N/A
	2-FG	0.034	0.027	0.042	0.049
	Rotenone	0.069	0.091	0.048	0.751

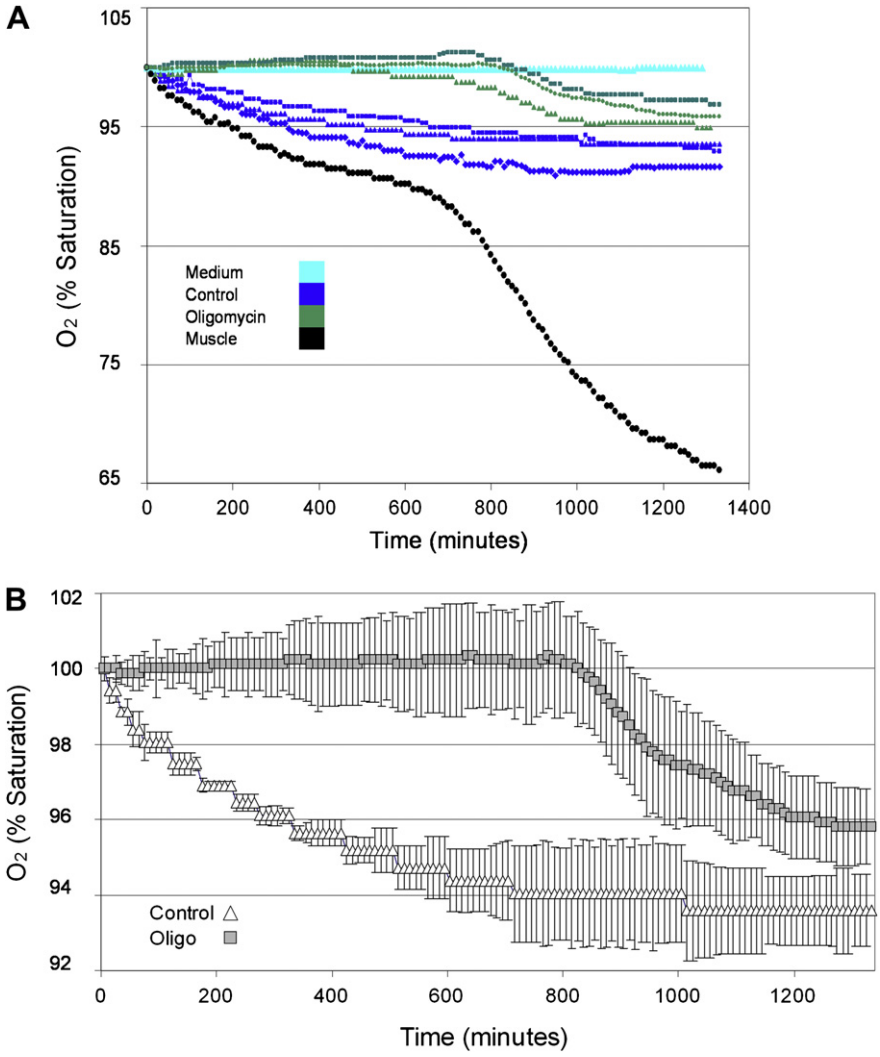

Fig. 3. Effect of oligomycin on oxygen consumption in cartilage explants. (A) Oxygen consumption by three untreated control cartilage samples (blue symbols) and three cartilage samples treated with 5 μg/ml oligomycin (green symbols). A negative control consisting of culture medium with no tissue (cyan symbols) and a positive control consisting of bovine skeletal muscle (black symbols) are also shown. (B) Group means and standard deviations at each time point are shown for untreated control samples and oligomycin treated samples.

blocked by rotenone[37]. The authors concluded that the mitochondrial ROS were important intracellular signals that affected gene and protein expression.

Evidence that oxidants play a supportive role in cartilage ATP synthesis was first reported by Lee and Urban. They demonstrated a negative Pasteur Effect whereby ATP and lactate production were severely impaired under anoxic conditions[1]. Subsequently, the authors tested the hypothesis that alternative electron acceptors could be substituted for oxygen itself[18]. In that experiment, glucose uptake and lactate production under anoxic conditions were restored to near normal values by methylene blue; 2,6-dichlorophenol-indophenol, or the iron (III) complex ferricyanide. The authors suggested that the glycolytic cycle was slowed by negative feedback from excessive accumulation of reduced metabolites in the absence of oxygen, a condition that exogenous oxidants alleviated. These observations raise the question of whether chondrocytes themselves generate intracellular oxidants as an adaptation to life in a state of intermittent moderate to severe hypoxia[41]. The rotenone effects we observed suggest that ET plays such role.

There were several limitations to this study. First, we cannot rule out the possibility that rotenone and/or 2-FG were selectively toxic to chondrocytes of the deeper zones, which were not visible by the *in situ* method used to evaluate viability. Furthermore, depth-dependent variation in mitochondrial function[25] may have led to depth-dependent inhibitor responses, a complexity that our bulk assays would have missed. Second, we chose to normalize our data to wet tissue mass, a reliable and well accepted measurement, but one that assumed consistent viable cell density. This was confirmed in part by our imaging studies, which showed that the average viability for all explants was 90% with a standard deviation only 6.3%. DNA was not used for normalization because neither fluorometric nor histologic assays discriminate between live cell DNA and the DNA of dead cells, which persists in the matrix for several days post-mortem[42].

A growing body of evidence implicates mitochondrial dysfunction in osteoarthritis[10–12,43–45] ET through complexes II and III of the mitochondrial ET chain is impaired while overall mitochondrial mass is increased in osteoarthritic cartilage. Larger numbers of swollen mitochondria with subnormal membrane potential are observed and these changes are associated with chondrocyte apoptosis[45]. Membrane depolarization brought on by calcium release provoked apoptosis in response to mechanical injury[10]. OA is associated with reduced expression of manganese-superoxide

dismutase (SOD2) in superficial zone chondrocytes[12] and oxidative damage to mitochondrial DNA[11]. In addition, previous work in our laboratory revealed that mitochondrial-derived ROS play a central role in acute chondrocyte death induced by mechanical injury to cartilage explants, an effect that may play an important role in the pathogenesis of post-traumatic OA. Taken together these findings indicate that mitochondria in particular and oxidative damage in general play key roles in the pathogenesis of OA.

In summary, our findings reveal a vital, if indirect role for mitochondria in chondrocyte energy production that focuses on mitochondrial ET as a source of oxidants to maintain redox balance under the predominantly reducing intra-articular environment. This suggests that subtle changes in mitochondrial ET function could undercut metabolic support for cartilage homeostasis long before overt cartilage degeneration is apparent. Such metabolic derangement, measurable as abnormalities in metabolite pools, may ultimately serve as a prognostic biomarker for OA. Our results also suggest that oxidants may be used therapeutically to restore redox balance and support cartilage metabolism under conditions, such as after articular injury when intra-articular oxygen reaches critically low levels[19,46,47].

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Contributions

Conception and design of the study: James A. Martin, Todd O. McKinley

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Competing interests

The authors have no financial or personal relationships with entities that could have influenced this work.

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